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INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI, BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DISSABS, DDFB, DDFU, DGENE, DRUGB, DRUGLAUNCH, ...' ENTERED AT 09:15:06 ON 30 NOV 2003

SEA (DNASE)	
28	FILE ADISCTI
7	FILE ADISINSIGHT
37	FILE ADISNEWS
466	FILE AGRICOLA
46	FILE ANABSTR
219	FILE AQUASCI
175	FILE BIOBUSINESS
133	FILE BIOCOMMERCE
15550	FILE BIOSIS
265	FILE BIOTECHABS
265	FILE BIOTECHDS
6045	FILE BIOTECHNO
1483	FILE CABA
3608	FILE CANCERLIT
18447	FILE CAPLUS
107	FILE CEABA-VTB
10	FILE CEN
55	FILE CIN
141	FILE CONFSCI
1	FILE CROPB
11	FILE CROPU
1128	FILE DISSABS
22	FILE DDFB
288	FILE DDFU
983	FILE DGENE
22	FILE DRUGB
27	FILE DRUGNL
569	FILE DRUGU
6	FILE DRUGUPDATES
92	FILE EMBAL
8261	FILE EMBASE
3794	FILE ESBIOBASE
176	FILE FEDRIP
33	FILE FROSTI
127	FILE FSTA
45112	FILE GENBANK
5	FILE HEALSAFE
492	FILE IFIPAT
702	FILE JICST-EPLUS
3	FILE KOSMET
5298	FILE LIFESCI
5	FILE MEDICONF
9695	FILE MEDLINE
47	FILE NIOSHTIC
60	FILE NTIS
35	FILE OCEAN
2413	FILE PASCAL
7	FILE PHAR
30	FILE PHARMAML
75	FILE PHIN
279	FILE PROMT
7221	FILE SCISEARCH

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             5060   FILE TOXCENTER
            10573   FILE USPATFULL
              303   FILE USPAT2
               14   FILE VETU
              380   FILE WPIDS
              380   FILE WPINDEX
L1               QUE (DNASE)
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     FILE 'CAPLUS, BIOSIS, USPATFULL, MEDLINE, EMBASE, SCISEARCH, BIOTECHNO,
     LIFESCI, TOXCENTER, ESBIOBASE, CANCERLIT, PASCAL' ENTERED AT 09:16:45 ON
     30 NOV 2003
L2          15211 S L1 AND (VARIANT OR MUTANT)
L3             28 S L2 AND (ASN74LYS OR N74K)
L4              7 DUP REM L3 (21 DUPLICATES REMOVED)
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=> d l4 ibib ab 1-7

L4 ANSWER 1 OF 7 USPATFULL on STN
ACCESSION NUMBER: 2002:307901 USPATFULL
TITLE: Human **DNase** I hyperactive **variants**
INVENTOR(S): Lazarus, Robert A., Millbrae, CA, UNITED STATES
Pan, Clark Qun, San Francisco, CA, UNITED STATES
PATENT ASSIGNEE(S): Genentech, Inc. (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002173025	A1	20021121
APPLICATION INFO.:	US 2001-5306	A1	20011107 (10)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1996-663831, filed on 14 Jun 1996, GRANTED, Pat. No. US 6391607
DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: GENENTECH, INC., 1 DNA WAY, SOUTH SAN FRANCISCO, CA, 94080
NUMBER OF CLAIMS: 20
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 4 Drawing Page(s)
LINE COUNT: 967
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to amino acid sequence **variants** of human **DNase** I that have increased DNA-hydrolytic activity. The invention provides nucleic acid sequences encoding such hyperactive **variants**, thereby enabling the production of these **variants** in quantities sufficient for clinical use. The invention also relates to pharmaceutical compositions and therapeutic uses of hyperactive **variants** of human **DNase** I.

L4 ANSWER 2 OF 7 USPATFULL on STN
ACCESSION NUMBER: 2002:116043 USPATFULL
TITLE: Human **DNase** I hyperactive **variants**
INVENTOR(S): Lazarus, Robert A., Millbrae, CA, United States
Pan, Clark Qun, San Francisco, CA, United States
PATENT ASSIGNEE(S): Genentech, Inc., South San Francisco, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6391607	B1	20020521
APPLICATION INFO.:	US 1996-663831		19960614 (8)

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Achutamurthy, Ponnathapu
ASSISTANT EXAMINER: Rao, Manjunath N.
LEGAL REPRESENTATIVE: Johnston, Sean A., Evans, David W
NUMBER OF CLAIMS: 10
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 4 Drawing Figure(s); 4 Drawing Page(s)
LINE COUNT: 1067
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to amino acid sequence **variants** of human **DNase** I that have increased DNA-hydrolytic activity. The invention provides nucleic acid sequences encoding such hyperactive **variants**, thereby enabling the production of these **variants** in quantities sufficient for clinical use. The invention also relates to pharmaceutical compositions and therapeutic uses of hyperactive **variants** of human **DNase** I.

L4 ANSWER 3 OF 7 USPATFULL on STN
ACCESSION NUMBER: 2001:205595 USPATFULL

TITLE: Human **DNase** I **variants**
INVENTOR(S): Lazarus, Robert A., Millbrae, CA, United States
Shak, Steven, Burlingame, CA, United States
Ulmer, Jana S., San Rafael, CA, United States
PATENT ASSIGNEE(S): Genentech, Inc. (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2001041360	A1	20011115
	US 6348343	B2	20020219
APPLICATION INFO.:	US 2001-796774	A1	20010228 (9)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1997-929995, filed on 15 Sep 1997, ABANDONED Continuation of Ser. No. US 1995-540527, filed on 10 Oct 1995, ABANDONED Continuation-in-part of Ser. No. US 1996-403873, filed on 24 Mar 1996, ABANDONED Continuation-in-part of Ser. No. WO 1995-US2366, filed on 24 Feb 1995, UNKNOWN
DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: Attn: David W Evans, GENENTECH, INC., 1 DNA Way, South San Francisco, CA, 94080-4990
NUMBER OF CLAIMS: 22
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 13 Drawing Page(s)
LINE COUNT: 1207
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to amino acid sequence **variants** of human **DNase** I that have reduced binding affinity for actin. The invention provides nucleic acid sequences encoding such actin-resistant **variants**, thereby enabling the production of these **variants** in quantities sufficient for clinical use. The invention also relates to pharmaceutical compositions and therapeutic uses of actin-resistant **variants** of human **DNase** I.

L4 ANSWER 4 OF 7 CAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 1
ACCESSION NUMBER: 1999:593926 CAPLUS
DOCUMENT NUMBER: 131:319595
TITLE: Ca2+-dependent activity of human **DNase** I and its hyperactive **variants**
AUTHOR(S): Pan, Clark Q.; Lazarus, Robert A.
CORPORATE SOURCE: Department of Protein Engineering, Genentech, Inc., South San Francisco, CA, 94080, USA
SOURCE: Protein Science (1999), 8(9), 1780-1788
CODEN: PRCIEI; ISSN: 0961-8368
PUBLISHER: Cambridge University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB We have recently constructed hyperactive human **DNase** I **variants** that digest double-stranded DNA more efficiently under physiol. saline conditions by introducing pos. charged amino acids at eight positions that can interact favorably with the neg. charged DNA phosphates. In this study, we present data from supercoiled DNA nicking, linear DNA digestion, and hyperchromicity assays that distinguish two classes of **DNase** I hyperactive **variants** based upon their activity dependence on Ca2+. Class A **variants** are highly dependent upon Ca2+, having up to 300-fold lower activity in the presence of Mg2+ alone compared to that in the presence of Mg2+ and Ca2+, and include Q9R, H44K, and T205K, in addn. to wild-type **DNase** I. In contrast, the catalytic activity of Class B **variants**, which comprise the E13R, T14K, **N74K**, S75K, and N110R hyperactive **variants**, is relatively Ca2+ independent. A significant proportion of this difference in Ca2+-dependent activity can be attributed to one of the two structural calcium binding sites in **DNase** I. Compared to wild-type, the removal of Ca2+ binding site 2 by alanine

replacements at Asp99, Asp107, and Glu112 decreased activity up to 26-fold in the presence of Mg2+ and Ca2+, but had no effect in the presence of Mg2+ alone. We propose that the rate-enhancing effect of Ca2+ binding at site 2 can be replaced by favorable electrostatic interactions created by proximal pos. charged amino acid substitutions such as those found in the Class B **variants**, thus reducing the dependence on Ca2+.

REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 5 OF 7 CAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 2

ACCESSION NUMBER: 1998:484316 CAPLUS
DOCUMENT NUMBER: 129:197961
TITLE: Improved potency of hyperactive and actin-resistant human **DNase** I **variants** for treatment of cystic fibrosis and systemic lupus erythematosus
AUTHOR(S): Pan, Clark Q.; Dodge, Tony H.; Baker, Dana L.; Prince, William S.; Sinicropi, Dominick V.; Lazarus, Robert A.
CORPORATE SOURCE: Department of Protein Engineering, Genentech, Inc., South San Francisco, CA, 94080, USA
SOURCE: Journal of Biological Chemistry (1998), 273(29), 18374-18381
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The ability of recombinant human **DNase** I (**DNase** I) to degrade DNA to lower mol. wt. fragments is the basis for its therapeutic use in cystic fibrosis (CF) patients and its potential use as a treatment for systemic lupus erythematosus (SLE). To increase the potency of human **DNase** I, we have generated and characterized three classes of **mutants**: (a) hyperactive **variants**, which have from one to six addnl. pos. charged residues (+1 to +6) and digest DNA much more efficiently relative to wild type, (b) actin-resistant **variants**, which are no longer inhibited by G-actin, a potent inhibitor of **DNase** I, and (c) combination **variants** that are both hyperactive and actin-resistant. For DNA scission in CF sputum where the DNA concn. and length are large, we measured a .apprx.20-fold increase in potency relative to wild type for the +3 hyperactive **variant** Q9R/E13R/**N74K** or the actin-resistant **variant** A114F; the hyperactive and actin-resistant combination **variant** was .apprx.100-fold more potent than wild type **DNase** I. For digesting lower concns. of DNA complexed to anti-DNA antibodies in human serum, we found a maximal enhancement of .apprx.400-fold over wild type for the +2 **variant** E13R/**N74K**. The +3 enzymes have .apprx.4000-fold enhancement for degrading moderate levels of exogenous DNA spiked into human serum, whereas the +6 enzyme has .apprx.30,000-fold increased activity for digesting the extremely low levels of endogenous DNA found in serum. The actin resistance property of the combination **mutants** further enhances the degree of potency in human serum. Thus, the human **DNase** I **variants** we have engineered for improved biochem. and pharmacodynamic properties have greater therapeutic potential for treatment of both CF and SLE.

REFERENCE COUNT: 32 THERE ARE 32 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 6 OF 7 CAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 3

ACCESSION NUMBER: 1997:510474 CAPLUS
DOCUMENT NUMBER: 127:118899
TITLE: The phosphorylation of bovine **DNase** I Asn-linked oligosaccharides is dependent on specific lysine and arginine residues
AUTHOR(S): Nishikawa, Atsushi; Gregory, Walter; Frenz, John;

Cacia, Jerry; Kornfeld, Stuart
CORPORATE SOURCE: Department Medicine, Washington University School Medicine, St. Louis, MO, 63110, USA
SOURCE: Journal of Biological Chemistry (1997), 272(31), 19408-19412
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The secretory glycoprotein, **DNase** I (I), acquires mannose 6-phosphate moieties on its Asn-linked oligosaccharides, indicating that it is a substrate for lysosomal enzyme N-acetylglucosamine-1-phosphotransferase (II). II recognizes a conformation-dependent protein determinant that is present in lysosomal hydrolases, but absent in most secretory glycoproteins. To identify the amino acid residues of I that are required for interaction with II, wild-type and **mutant** forms of bovine I were expressed in COS-1 cells and the extent of *oligosaccharide phosphorylation detd.* Phosphorylation of I oligosaccharides decreased from 12.6 to 2.3% when Lys-50, Lys-124, and Arg-27 were mutated to Ala, indicating that these residues are required for the basal level of phosphorylation. Mutation of Lys at other positions did not impair phosphorylation, demonstrating the selectivity of this process. When Arg-27 was replaced with a Lys, phosphorylation increased to 54%, showing that II prefers Lys to Arg residues. Mutation of Asn-74 to Lys also increased phosphorylation to 50.3%, and the double **mutant** (R27K/**N74K**) was phosphorylated 79%, equiv. to the values obtained with lysosomal hydrolases. Interestingly, Lys-27 and Lys-74 caused selective phosphorylation of the neighboring Asn-linked oligosaccharide. Finally, mutation of Lys-117 to Ala stimulated phosphorylation, demonstrating that some residues may be neg. regulators *of this process. It was concluded that selected Lys* and Arg residues on the surface of **DNase** I constitute the major elements in the II recognition domain present on this secretory glycoprotein.

L4 ANSWER 7 OF 7 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1997:400687 CAPLUS
DOCUMENT NUMBER: 127:132688
TITLE: Engineering hyperactive human **DNase** I with over 4000-fold higher activity in human serum
AUTHOR(S): Pan, Clark O.; Dodge, Tony H.; Baker, Dana L.; Sinicropi, Dominick; Lazarus, Robert A.
CORPORATE SOURCE: Department of Protein Engineering, Genentech, Inc., South San Francisco, CA, 94080, USA
SOURCE: Protein Engineering (1997), 10(Suppl.), 88
CODEN: PRENE9; ISSN: 0269-2139
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The ability of recombinant human **DNase** I to degrade DNA represents an important therapy for cystic fibrosis and a potential therapy for systemic lupus erythematosus (SLE) patients. In an attempt to improve its clin. benefits, the authors have engineered hyperactive human **DNase** I **variants** by altering its functional activity from the native single-stranded nicking pathway to a much more efficient double-stranded cutting pathway. In human serum, as high as 4200-fold greater activity than the wildtype **DNase** I was detected for a **mutant**, E13R:**N74K**:T205K. The improvement was larger in serum than in buffer, perhaps because of the presence of other DNA-binding proteins in serum that inhibit wildtype activity more than those of the **variants**. In addn., the hyperactive **variants** were more resistant to salt inhibition than the wildtype **DNase** I.

NCBI PubMed National Library of Medicine

Entrez PubMed Nucleotide Protein Genome Structure PMC Journals

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Search	Most Recent Queries	Time	Result
#22	Search **human DNase variant** Field: **Title/Abstract,** Limits: **Publication Date from 1970 to 1996**	09:35:24	6
#21	Search **human DNase variant** Field: **Title,** Limits: **Publication Date from 1970 to 1996**	09:35:15	0
#20	Search **(human DNase variant) OR (human DNase mutant)** Field: **Title,** Limits: **Publication Date from 1970 to 1996**	09:34:57	0
#11	Search **#5AND#7AND#8** Field: **Title/Abstract,** Limits: **Publication Date from 1970 to 1996**	09:33:51	103
#10	Search **#5 AND #7 AND #8** Field: **Title/Abstract,** Limits: **Publication Date from 1970 to 1996**	09:26:38	0
#9	Search **#5 AND #8** Field: **Title/Abstract,** Limits: **Publication Date from 1970 to 1996**	09:26:14	0
#8	Search **N74 OR N74K OR Asn74lys** Field: **Title/Abstract,** Limits: **Publication Date from 1970 to 1996**	09:25:34	3
#7	Search **variant or mutant** Field: **Title/Abstract,** Limits: **Publication Date from 1970 to 1996**	09:24:48	93238
#5	Search **human DNase** Field: **Title,** Limits: **Publication Date from 1970 to 1996**	09:23:55	103
#3	Search **#1 AND #2** Field: **Title/Abstract,** Limits: **Publication Date from 1970 to 1996**	09:22:58	45
#2	Search **variant OR mutant** Field: **Title/Abstract,** Limits: **Publication Date from 1970 to 1996**	09:22:40	93238
#1	Search **human DNase** Field: **Title/Abstract,** Limits: **Publication Date from 1970 to 1996**	09:22:19	1420

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Term: L1 same (Q9R or E13K or T14K or T14R or H44K or H44R or N74K or N74R or S75K or T205K or T205R)

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L4	L3 same (Q9R or E13K or T14K or T14R or H44K or H44R or N74K or N74R or S75K or T205K or T205R)	4	L4
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1. Document ID: US 20020173025 A1

L5: Entry 1 of 4 File: PGPB Nov 21, 2002

PGPUB-DOCUMENT-NUMBER: 20020173025
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020173025 A1

TITLE: Human DNase I hyperactive variants

PUBLICATION-DATE: November 21, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Lazarus, Robert A.	Millbrae	CA	US	
Pan, Clark Qun	San Francisco	CA	US	

US-CL-CURRENT: 435/199; 435/320.1, 435/325, 435/6, 435/69.1, 536/23.2

2. Document ID: US 20010041360 A1

L5: Entry 2 of 4 File: PGPB Nov 15, 2001

PGPUB-DOCUMENT-NUMBER: 20010041360
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20010041360 A1

TITLE: Human DNase I variants

PUBLICATION-DATE: November 15, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Lazarus, Robert A.	Millbrae	CA	US	
Shak, Steven	Burlingame	CA	US	
Ulmer, Jana S.	San Rafael	CA	US	

US-CL-CURRENT: 435/196; 424/94.6, 536/23.2

3. Document ID: US 6391607 B1

L5: Entry 3 of 4 File: USPT May 21, 2002

US-PAT-NO: 6391607

DOCUMENT-IDENTIFIER: US 6391607 B1

TITLE: Human DNase I hyperactive variants

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KMC | Image

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4. Document ID: US 6348343 B1

L5: Entry 4 of 4 File: USPT Feb 19, 2002

US-PAT-NO: 6348343
DOCUMENT-IDENTIFIER: US 6348343 B1

TITLE: Human DNase I variants

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KMC | Image

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